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TITLE: Role of Cholesterol Homeostasis in Lupus Pathogenesis

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Improper regulation of a specific immune cell called a B cell has been long linked to the development of SLE. A subtype of B cells, termed Autoimmune/Age-associated B cells (ABC), has recently been shown to play a major role in SLE because of their ability to be major producers of the proteins, also known as autoantibodies, that can cause damage in lupus. Expansion of ABCs in SLE is greater in African-American patients and correlates with disease activity and clinical manifestations. The environmental triggers that promote the accumulation of ABCs in lupus patients are largely unknown. Our laboratory has found that the expansion of ABCs in mice is controlled by a small family of two molecules. We have found that deleting both of these molecules in mice (leading to a Double Knock-out=DKO) leads to the spontaneous development of lupus in mice that shares many features with the human disease including the fact that the disease primarily affects female mice. We have recently found that manipulating cholesterol levels in these mice can promote the expansion of ABCs and affect the extent of inflammation in different organs in these mice. In this proposal we will investigate the hypothesis that alterations in cholesterol, as could be driven by a Western-diet rich in cholesterol, can affect the accumulation of ABCs and the ability of inflammatory cells to target specific organs and thus contribute to the heterogeneity of SLE.

15. SUBJECT TERMS None listed.					
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1. INTRODUCTION:

A major roadblock to our ability to develop novel treatments for Systemic Lupus Erythematosus (SLE) is the significant heterogeneity that accompanies this disease. The complex cross-talk between an individual's genetic predisposition and exposure to environmental factors like diet is likely to be a major contributor to this heterogeneity. Autoimmune/Age-associated B cells (ABCs) are a novel B cell subset, which exhibit a unique phenotype and preferentially expand in SLE patients. ABCs are major producers of autoantibodies, and their expansion in SLE correlates with disease activity and clinical manifestations like kidney disease. The environmental triggers that promote the generation, function, and differentiation of ABCs in autoimmune settings are largely unknown. Our laboratory previously isolated a protein termed Def6, which exhibits significant homology to only one other protein, SWAP-70. Importantly, mice lacking both Def6 and SWAP-70 (Double-knockout=DKO mice) develop SLE-like disease, which shares several key clinical features with human SLE including its sex-bias. We have previously shown that ABC formation is enhanced in DKO mice. In this proposal we are testing the hypothesis that alterations in lipid homeostasis, such as those promoted by a Western diet, can modulate the accumulation and function of ABCs and impact autoAb responses and end-organ inflammation to a different extent based on host-specific factors and thus contribute to SLE heterogeneity.

2. KEYWORDS:

WD=Western Diet, LDLR= Low-density lipoprotein receptor, ABCs= Autoimmunity/Age-associated B cells, DN2= Double negative 2 B cells, DKO= Double knock-out (mice lacking both Def6 and SWAP-70)

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: To delineate the mechanisms by which a WD promotes the expansion and differentiation of ABCs

Major Task 1: To assess the transcriptional and epigenetic effects exerted on ABCs by ingestion of a WD (Yr.1)

Subtask 1: RNASEQ chow vs WD

Subtask 2: ATAC-seq chow vs WD

Milestone(s) Achieved: The studies have established that the presence/absence of a Western diet alters the molecular profile of ABCs.

Major Task 2: To delineate the role of key regulators of cholesterol homeostasis in ABCs (Yr. 2)

Subtask 1: Analysis of CD23cre SREBP2$^{fl/fl}$DKO post chow or WD-in vivo experiments

Establishment of requirement for SREBP2 in WD- driven effects on humoral autoimmunity

Milestone(s) Achieved: The studies have established a key role for SREBP2 in humoral autoimmunity in the presence and absence of a Western diet.

Specific Aim 2: To dissect the mechanisms by which alterations in lipid homeostasis modulate end-organ inflammation in lupus (Yr 3)

Major Task 3: To evaluate the effects of dietary influences on the development of atherosclerosis in DKO mice in the presence/absence of genetic manipulations of the LDLR

Milestone(s) Achieved: The studies have demonstrated that DKO mice lacking the LDLR and fed a WD are protected from the development of atherosclerosis.

Major Task 4: To define the cellular and molecular composition of the inflammatory infiltrates in end- organs of DKO mice with alterations in lipid homeostasis.

Milestone(s) Achieved: The studies have shown that alterations in lipid homeostasis modulate the molecular profile of immune cells in end-organs.

What was accomplished under these goals?

Aim 1.1: To assess the transcriptional and epigenetic effects exerted on ABCs by ingestion of a WD. Like human SLE, the expansion and production of autoAbs by DKO ABCs exhibits a striking sex-bias and is primarily observed in DKO female mice (Fig. 1). Remarkably, feeding a WD to DKO males promotes the accumulation of ABCs and autoAb production suggesting that the ability of these cells to accumulate and secrete autoAbs can be regulated not only by genetic and sex-specific factors but also by dietary influences. The major activities for the Yr 1 reporting period were to utilize unbiased genome-wide approaches to gain insights into the mechanisms that underlie the ABC-promoting effects of a WD. The specific objectives were to employ RNA-seq and ATAC-seq to investigate the transcriptional and epigenetic effects exerted on ABCs by ingestion of a WD. To investigate this question, we have performed RNA-seq on ABCs sorted from DKO males or females that were either fed chow or a WD, which was started at approximately 8 wks and continued for 12 wks. Sex-matched and diet-matched wt controls were included as controls for the diet. Analysis of the RNAseq experiments has been completed and has revealed the following: 1) Feeding a WD to DKO females resulted in 576 upregulated and 678 downregulated genes (log_2FC .6; $p<.05$; Fig 1); 2) Feeding a WD to DKO males resulted in 178 upregulated and 92 downregulated genes (log_2FC .6; $p<.05$; Fig. 2); 3) Feeding a WD to either DKO females or DKO males led to upregulation of a common set of pathways. Importantly both DKO females and DKO males fed a WD upregulated TNFα signaling and NF-κB (Red rectangles) and thus exhibited an increased inflammatory profile (Fig. 3); 4) DKO males but not DKO females fed a WD upregulated pathways involved in B cell receptor signaling (blue rectangle, Fig 3) suggesting indeed that feeding a WD can promote enhanced sensitivity of ABCs to BCR engagement and thus increased antigenic responsiveness. This analysis has thus uncovered similarities but also crucial transcriptional differences between ABCs derived from DKO males fed chow versus those obtained from DKO males fed a WD and has revealed that these distinctions/similarities reflect the differential expression of a selected group of pathways. A separate cohort of DKO males and DKO females that were either fed chow or a WD, which was started at approximately 8 wks and continued for 12 wks was also set-up for ATAC-seq. Sex-matched and diet-matched wt controls were included as controls for the diet. ABC cells have been sorted and processed for ATAC-seq and ATAC-seq has demonstrated that these transcriptional differences are accompanied by differences in the chromatin landscape of these cells with differences observed between DKO females fed a WD (termed HFD in Fig. 4) or chow (termed control in Fig.4). Differences were also observed in the ATAC-seq results of DKO males and females fed a WD/HFD (Fig. 5) in line with the transcriptional differences observed by RNA-seq.

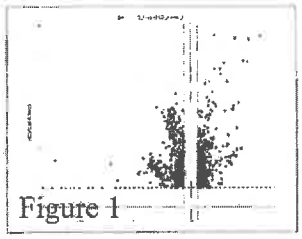
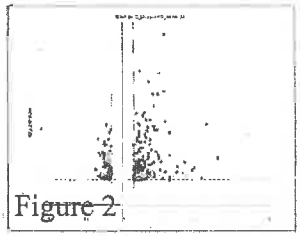


Figure 1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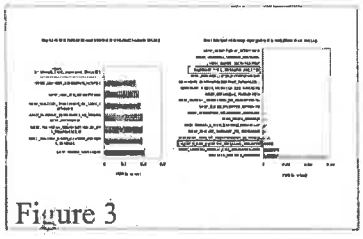
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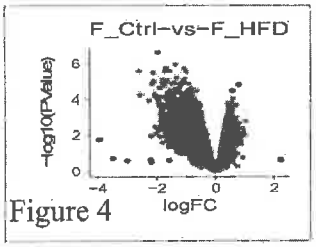

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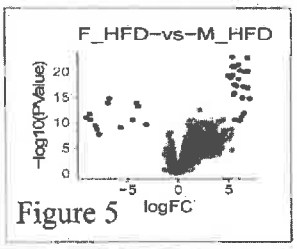

Figure 5

Aim 1.2: To delineate the role of key regulators of cholesterol homeostasis in ABCs. Sterol regulatory element-binding protein 2 (SREBP2) is the major transcriptional regulator of sterol synthesis and directly induces the expression of enzymes involved in the mevalonate pathway. In response to cholesterol starvation, SREBP2 is activated by a complex process involving its transport from the ER to the Golgi where it is cleaved followed by the translocation of transcriptionally active fragments to the nucleus. Interestingly, we have recently observed that, in B cells, the activity of SREBP2 can be regulated by its interaction with IRF family members. The major activities for the Yr 2 reporting period were to investigate the role of SREBP2 in ABC function and whether this role would be altered by ingestion of a WD. To this end we took advantage of SREBP2-deficient DKO mice, which we had previously generated by crossing SREBP2fl/fl mice with DKO mice and then with CD23Cre mice to generate CD23-Cre+ SREBP2fl/flDKO female and male mice. These mice were fed either chow or a WD, which was started at approximately 8 wks and continued for 12 wks as in Aim 1 followed by an extensive analysis of the effects of these manipulations on ABC generation and differentiation in vivo. A detailed assessment of all key B cell populations in CD23-Cre+ SREBP2fl/flDKO female mice (Fig. 6 and data not shown) demonstrated that expression of SREBP2 regulates the expansion of ABCs and even more profoundly their ability to differentiate into a pre-GC B cell population (CD11c+ GCB cells) and subsequently into classical GCs. Differentiation toward PB/PCs was instead not significantly affected suggesting that SREBP2 primarily controls the GC route of ABC differentiation but not the extrafollicular (EF) route. These effects were lessened by administration of a WD consistent with the idea that SREBP2 controls the production of endogenous cholesterol by B cells and that this step can be bypassed, albeit not completely, by providing exogenous cholesterol. Interestingly, administration of a WD was less effective at ameliorating the GC defect observed in the absence of SREBP2 suggesting that the compensatory mechanisms function in a B-cell stage specific manner. A similar analysis in CD23-Cre^{+}SREBP2$^{fl/fl}$DKO male mice (Fig. 7 and data not shown) indicated a greater impact of SREBP2 deletion on ABC differentiation and GC formation under a WD suggesting that the ABC compartment of males may be less able to compensate for the absence of SREBP2 and that in males SREBP2 may control both GC and EF routes of differentiation. An extensive serological analysis (Fig. 8 and Fig. 9 and data not shown) demonstrated sex-specific differences in the production of autoantibodies in the absence of SREBP2 with stronger effects in the males consistent with the greater role of SREBP2 in regulating the ABCs in males than females. We also observed an unexpected increase in the production of SmRNP autoantibodies in males suggesting that, in males, SREBP2 controls not only the ABCs but also the function of specific subsets of PB/PCs.

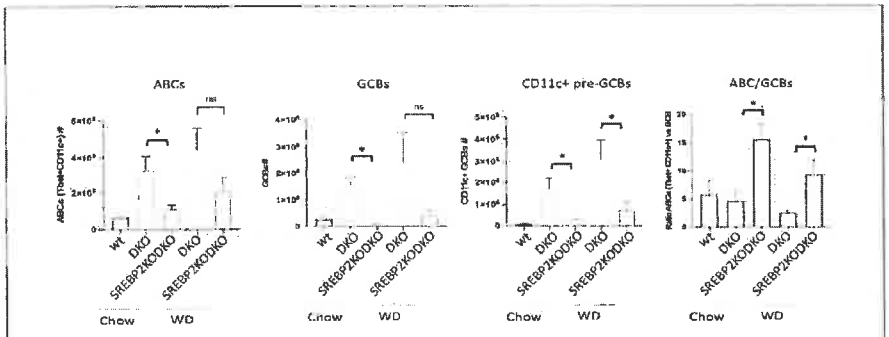

Figure 6
(females)

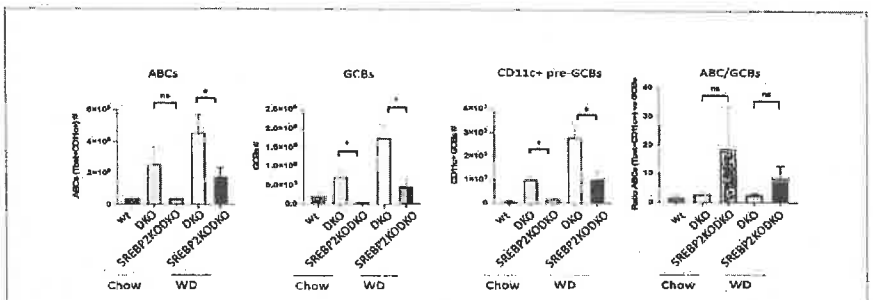

Figure 7
(males)

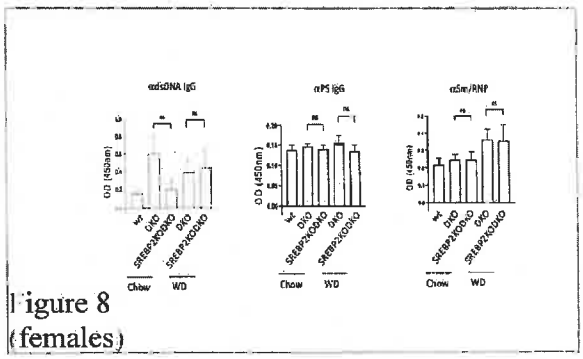

Figure 8
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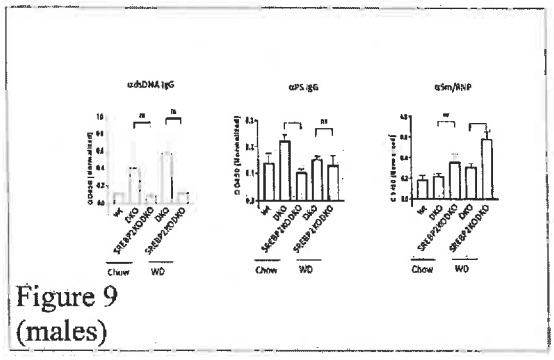
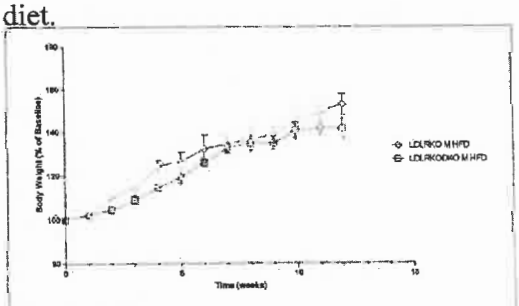


Figure 9
(males)

Aim 2.1. To investigate the mechanisms by which a Western life-style can impact the development of atherosclerosis in lupus-prone mice. Compared with age-and sex-matched controls, SLE patients exhibit an increased risk for cardiovascular events despite similar profiles of traditional CV risk factors. The mechanisms that predispose a subset of SLE patients to CVD are unclear. In these studies, we have employed DKO mice that either express or lack the LDL receptor to investigate the interplay between the chronic inflammatory milieu of lupus and dietary influences like a WD in modulating the development of atherosclerosis. Importantly, absence of the LDLR is known to lead to a lipid profile that closely resembles the profile observed in human subjects providing a clinically relevant setting. For these experiments, groups of female and male mice of the various genotypes (wt, DKO, LDLR-KO, and LDLRKO-DKO) were fed either chow or a WD for 12 weeks. To ensure reproducibility of the results separate cohorts of mice were set-up. No significant differences in body weight were observed amongst the various genotypes before, during, or after WD feeding in either males or females. In particular, male and female DKO mice lacking the LDLR exhibited similar weight gains as male and female mice lacking the LDLR on a nonautoimmune C57BL/6 background (LDLRKO) (Fig. 10-11). As expected only mice lacking the LDLR either in the presence (LDLRKODKO) or absence of the DKO background (LDLRKO) displayed increased plasma cholesterol levels (Fig. 12-13). The increase in plasma cholesterol levels was furthermore similar between LDLRKO and LDLRKODKO mice (Fig. 12-13). Despite similar increases in cholesterol levels upon feeding a WD, however, development of atherosclerosis was markedly decreased in LDLRKODKOs as compared to LDLRKO mice (Fig. 14-15). This effect was observed in both male and female mice (Fig. 14-15). Thus, surprisingly, absence of the LDLR in lupus prone DKO mice (i.e. LDLR-DKO mice) leads to a lower burden of atherosclerotic plaques upon feeding a WD.

To gain insights into this unexpected phenotype we investigated whether the interplay between the WD and the lupus-prone background would affect the production of antibodies directed not only against nuclear antigens like dsDNA but also of antibodies against phospholipids and oxidation-specific epitopes of low-density lipoprotein (LDL), such as IgM anti-malondialdehyde-modified LDL (*MDA-LDL*), which have been deemed to have atheroprotective roles. Interestingly, as compared to LDLRKO mice, both female and male LDLRKODKO mice exhibited a greater production of antibodies directed against MDA-LDL (Fig. 16-17) suggesting that production of these antibodies can contribute to the marked decrease in atherosclerosis in the LDLRKODKO mice upon WD feeding. These findings thus support the notion that differential production of atheroprotective antibodies can contribute to the heterogeneity of SLE patients by lessening the development of CVD despite exposure to chronic inflammation and environmental factors such as a WD diet.



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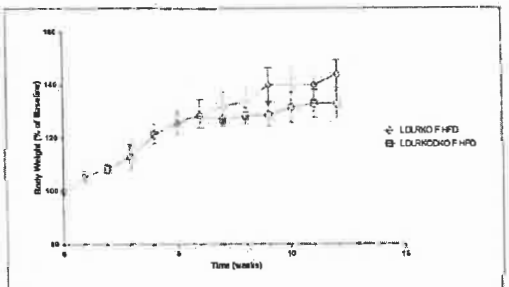

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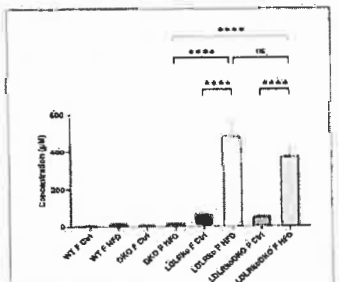

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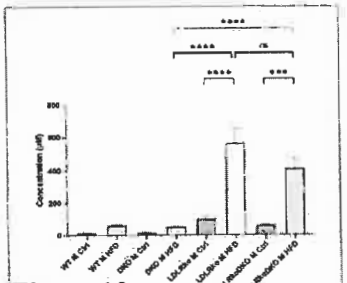

Figure 13
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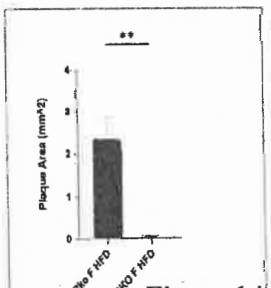

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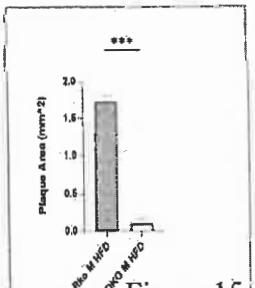

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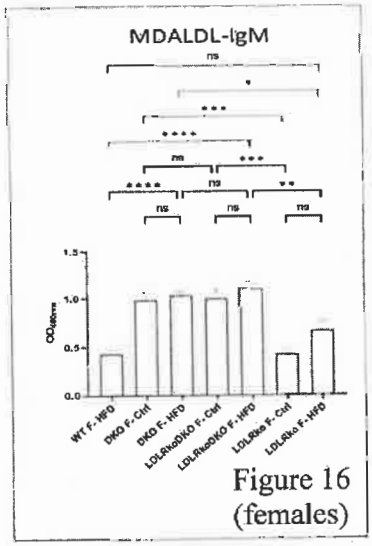

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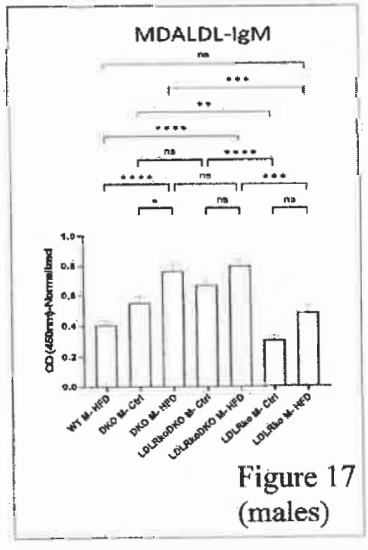
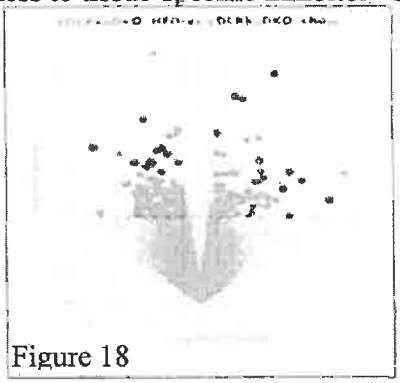


Figure 17
(males)

Aim 2.2. To define the cellular and molecular composition of the inflammatory infiltrates in end-organs of lupus-prone DKO mice with alterations in lipid homeostasis. To assess whether the interplay between lupus and dietary influences like a WD also modulates the chronic inflammatory infiltrates that accumulate in end-organs like the kidneys, the molecular profile of the infiltrates that accumulate in the kidneys was assessed in female lupus mice that were fed chow or a WD for 12 weeks. For these experiments, immune cell populations from DKO and LDLRKODKO mice were sorted from the kidneys and subjected to RNASeq. Unfortunately, the quality of the RNA obtained from several immune cell subsets was markedly affected by the tissue processing required to extract cells from the renal parenchyma impacting our ability to perform RNAseq on these cells. We were, however, able to obtain sufficient amounts of high-quality RNA from sorted ABCs for the studies. The high levels of cholesterol resulting from feeding a WD (termed HFD in the figures) in DKO mice lacking the LDLR markedly affected the transcriptional profile of the ABCs that infiltrated the kidneys as demonstrated by comparing LDLRKODKO fed a WD vs LDLRKODKO fed chow (Fig. 18) or LDLRKODKOs fed a WD vs DKOs fed a WD (Fig. 19). Interestingly, an analysis of pathways altered by exposure to a WD by performing GSEA demonstrated that high levels of cholesterol upregulated several pathways that promoted the proliferation of ABCs and their metabolic fitness (Fig 20-21) while downregulating pathways related to tissue-specific cues provided by the extracellular matrix and hypoxic environment (Fig. 22-23) suggesting that exposure to high-levels of cholesterol can promote the expansion of immune cells within the tissue and lessen their responsiveness to tissue-specific inhibitory signals.



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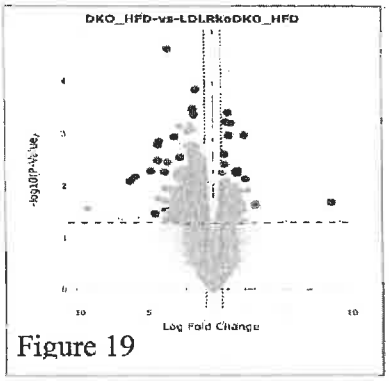

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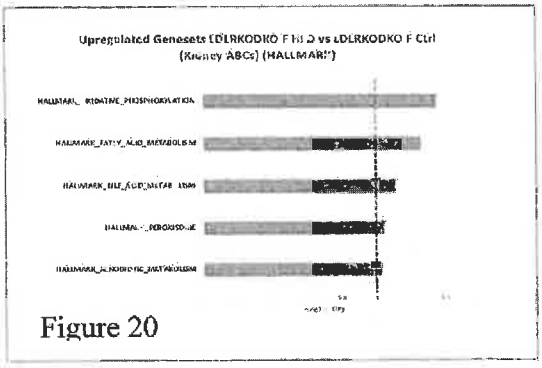

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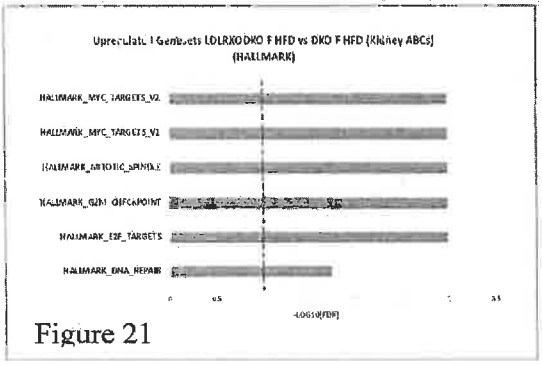

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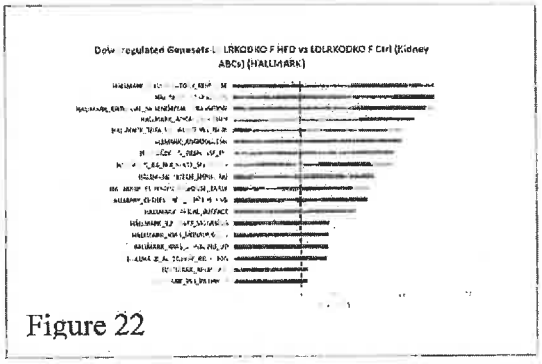

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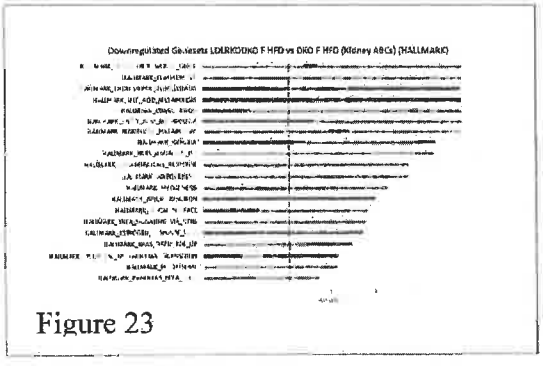

Figure 23

What opportunities for training and professional development has the project provided?

The PI attended the Lupus 21st Century meeting and the Annual College of Rheumatology meeting to catch up with recent advances in lupus research. The PI and Dr. Gupta also attended Research-in-progress meetings at the Hospital for Special Surgery on a regular basis and closely communicate with other scientists in the field of autoimmunity research.

How were the results disseminated to communities of interest?

Nothing to Report at present. All the data obtained are now being assembled in a manuscript

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. **CHANGES/PROBLEMS:**

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Nothing to report

Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Dr. Alessandra Pernis*
Project Role:	*PD/PI*
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-8259-1446
Nearest person month worked:	*0.60 calendar months effort, 0.36 calendar months' salary*
Contribution to Project:	*Dr. Pernis has guided the studies as well as heledp with the analysis of the results and is assisting in the preparation of manuscripts. She has trained in cellular immunology as well as biochemistry and molecular biology.*
Funding Support:	*This award*
Name:	*Dr. Sanjay Gupta*
Project Role:	*Postdoctoral*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*6.0 effort*
Contribution to Project:	*Dr. Gupta worked with Dr. Pernis and helped perform the analysis described in Major Tasks 3 and 4*
Funding Support:	*This award*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES:

Not applicable

Transition Plan Questionnaire

Directions: Please answer all questions that apply for each product under development. Please fill out one document per product. ***This is not an application for funding; however, answers will help us understand the outcomes and products from your award.***

1. After the award closes, would you be willing to periodically provide voluntary information (via email) regarding the project status (i.e. where the research is headed)? **Yes** [✓] or **No** [ ]
These responses will help CDMRP demonstrate the return on its investments and will help demonstrate that the CDMRP is a responsible and successful steward of federal research funding.

2. What **conclusion(s)** does your final data support?

1) These studies have shown that feeding a Western-diet can lead to key molecular differences in a B cell population (the ABCs) and that some of these differences can be controlled in a sex-specific manner
2) These studies have also shown that known regulators of cholesterol biosynthesis can also exert important roles in regulating the function of B cell populations and humoral responses.
3) These studies also surprisingly suggest that higher production of atheroprotective antibodies in response to high-levels of cholesterol could help lessen the development of atherosclerosis despite the presence of chronic inflammation. These results could contribute to the known heterogeneity of SLE
4) These studies also suggest that exposure to high-levels of cholesterol could worsen damage in other organs like the kidney by promoting the expansion of immune cells within the tissue and lessening their responsiveness to tissue-specific inhibitory signals.

3. Will you/have you applied for/obtained follow-on-funding for this project? **If yes**, please list (a) funding organization, (b) total budget requested/obtained, and (c) title of the funded proposal. *This information will be recorded as an outcome to this award.*

Have not yet applied but will be applying to the NIH for further funding to follow-up on the novel results obtained

4. What will be **the next step(s)** for this project?

1) Further testing the role of crucial transcriptional regulators in modulating autoimmune responses in SLE.
2) Extend the findings to SLE patients.

5. How would you classify your **lead candidate product**? e
(a) Therapeutic (Small Molecule, Biologic, Cell/Gene Therapy): Please choose, if applicable
(b) Diagnostic
(c) Device
(d) Research Tool to Address a Research Bottleneck
(e) Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.)
(f) Other - Please Specify:

6. How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?

The knowledge derived from these studies has enabled us to characterize key steps by which environmental factors like diet can influence SLE heterogeneity. In particular these studies once extended to SLE patients could lead to novel and more targeted therapeutic approaches for SLE.

7. Therapy / Product Development, Transition Strategies, and Intellectual Property

Describe the steps and relevant strategies required to move the candidate product (knowledge or tangible) to the next phase of development and/or commercialization. Please address any issues with intellectual property.

PIs are encouraged to explore the technical requirements and the current regulatory strategies involved in product development as well as to work with their organization's Technology Transfer Office (or equivalent regulatory/legal office), federal/international regulatory experts, to develop the transition plan and to explore developing relationships with industry, DoD advanced developers (e.g. USAMMDA), and/or other funding agencies to facilitate moving the product into the next phase.

Not applicable

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause (See Instructions on Reverse Side)

Form Approved
OMB No 9000-0095
Expires Aug 31, 2001

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THIS ADDRESS. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT (X one)
Alessandra Pernis	W81XWH2010372	Hospital for Special Surgery	W81XWH2010372	a. INTERIM X b. FINAL
b. ADDRESS *(Include ZIP Code)*	d. AWARD DATE *(YYMMDD)*	b. ADDRESS *(Include ZIP Code)*	d. AWARD DATE *(YYMMDD)*	4. REPORTING PERIOD *(YYMMDD)*
535 E. 70th St. NY, NY 10021	2020/09/15	535 E. 70th St. NY, NY 10021	2020/09/15	a. FROM 2020/09/15 b. TO 2023/09/14

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO E REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None" so state) NONE.*

NAME(S) OF INVENTOR(S) (Last, First, M) a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS d. (1) UNITED STATES (a) YES	(b) NO	(2) FOREIGN (a) YES	(b) NO	CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER (x) e. (a) YES	(b) NO
None to report	N/A	N/A						

f. EMPLOYER OF INVENTOR'S NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1)(a) NAME OF INVENTOR *(Last, First, M)*	(2)(a) NAME OF INVENTOR *(Last, First, M)*	(1) TITLE OF INVENTION	(2) FOREIGN COUNTIRES OF PATENT APPLICATION
None		N/A	
(b) NAME OF EMPLOYER	(b) NAME OF EMPLOYER		
(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*	(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*		

SECTION II - SUBCONTRACTS *(Containing a "Patents Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None" so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS *(Include ZIP Code)* b.	SUBCONTRACT NUMBER(S) c.	DRAR "PATENT RIGHTS" d. (1) CLAUSE NUMBER	(2) DATE *(YYMMDD)*	DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(s) e.	f. SUBCONTRACT DATES *(YYMMDD)* (1) AWARD	(2) ESTIMATED COMPLETION
None.							

SECTION III - CERTIFICATION

7. CERTICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if (X as appropriate))* | Small Business or | X | Non-Profit organization

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. Name of Authorized Contractor/Subcontractor Official *(Last, First, Middle Initial)*	b. TITLE	c. SIGNATURE	d. DATE SIGNED
Quigley, Pamela A.	Director Sponsored Programs Hospital for Special Surgery	Pamela Quigley	2024/01/09

FEDERAL FINANCIAL REPORT

Follow Form Instructions

1. Federal Agency and Organizational Element to Which Report is Submitted	2. Federal Grant or Other Identifying Number Assigned by Federal Agency	Page of
USA MED RESEARCH ACQ ACTIVITY - DEPARTMENT OF DEFENSE	W81XWH2010372	1 pages

3. Recipient Organization (Name and complete address, including ZIP code)

HOSPITAL FOR SPECIAL SURGERY
535 East 70th Street, New York, NY 10021

4a. DUNS Number	4b. EIN	5. Recipient Account Number or Identifying Num	6. Report Type	7. Basis OF Accounting
622146454	1131624135A1	3734800 PERNIS	Quarterly Semi-Annual Annual X Final	Cash X Accrual

8 Project / Grant Period (Month, Day, Year)		9. Reporting Period End Date (Month, Day, Year)
From: SEPTEMBER 15, 2020	To: SEPTEMBER 14, 2023	SEPTEMBER 14, 2023

10. Transactions:	Cumulative
Federal Cash	
a. Cash Receipts	499,041.91
b. Cash Disbursements	526,000.00
c. Cash on Hand (line a minus b)	26,958.09
(Use lines d - o for single grant reporting)	
Federal Expenditures and Unobligated Balance:	
d. Total Federal Funds Authorized	526,000.00
e. Federal Share of Expenditures	526,000.00
f. Federal share of unliquidated obligations	0
g. Total federal share (Sum of lines e and f)	526,000.00
h. Unobligated balance of Federal funds (Line d minus g)	0.00
Recipient Share:	
i. Total Recipient share required	
j. Recipient Share of Expenditures	
k. Remaining recipient share to be provided (line i minus j)	
Program Income:	
l. Total Federal Share of program income earned	
m. Program income expended in accordance with the deduction alternative	
n. Program income expended in accordance with the addition alternative	
o. Unexpended program income (line l minus line m and line n)	

11. Indirect Expense	a. Type	b. Rate	c. Period From	Period To	d. Base	e. Amount Charged	f. Federal Share
	Predetermined	78%	SEPTEMBER 15, 2020	SEPTEMBER 30, 2023	$298,296	$ 226,704	$ 226,704
				Totals	$298,296	$ 226,704	$ 226,704

12. Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.

13. Certification: I certify to the best of my knowledge and belief that this report is correct and completed and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.

Typed or Printed Name and Title of Authorized Certifying Official	c. Telephone (Area code, number and extension)
George Spencer - Controller	212 606 - 1696
	d. Email Address
Signature of Authorized Certifying Official	e. Date Report Submitted (Month, Day, Year)
George Spencer	1/11/2024

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